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AMINO ACIDS OF FULVIC AND HUMIC ACIDS  
IN CERTAIN TYPES OF SOILS

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## AMINO ACIDS OF FULVIC AND HUMIC ACIDS IN CERTAIN TYPES OF SOILS

N. G. Zyrin, M. F. Ovchinnikova and D. S. Orlov

**ABSTRACT.** One-way paper chromatography is used to study the relationships between various amino acids and groups of acids in various soils under various plant covers. Seventeen amino acids were determined. The content of amino acids correlated well with condensation of the humic materials. The relative contents of amino acids were found to be quite consistent, varying mostly with plant types; the relative contents of types of amino acids was determined by microbiological activity in the soil, not soil type. The nitrogen in the amino acids was 30-50% of the total hydrolyzable forms present in the soil.

According to modern conceptions, amino acids participate continually in the processes of humification and are one of the most important sources of nitrogen. Nitrogen is included in humic acids and fulvic acids as heterocyclic compounds [1] and in the composition of amino groups of the relatively simple amino acids, amino sugars, etc.; a continuous cycle of biochemical conversions leads to the fixation of the nitrogenous components in the nonhydrolyzable portions of the humus materials. The mechanism of these processes is quite complex and varied. The amino acids, entering the soil in the composition of organic remains, participate in processes of deamination and transamination [2], and interact with the mineral and organic compounds in the soil; certain free amino acids may be one of the direct sources of nitrogen for plants [3-6]. Amino acids included in the humus materials take on a certain stability and become less available to micro-organisms and plants. However, information on the nature and degree of the participation of amino acids in humus formation is as yet too limited to make a judgment concerning their role in the nitrogen supply of plants and the formation of humus material. /108<sup>1</sup>

Various authors have published results of chromatographic analysis of the amino acid composition of soil hydrolysates [7-13] and hydrolysates of humic acids from various types of soils: podzolic, chernozem, sierozem, peaty, brown forest soil and soil based on volcanic rock [2, 14-18].

Some investigators have studied the amino acid composition of plasma proteins [7], humus-like products of the vital activity of fungi [14, 19], as well as fulvic acids [8, 14].

In spite of the differences in the conditions of chromatography, these authors have found qualitatively similar amino acid compositions in the

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<sup>1</sup> Numbers in the margin indicate pagination in the foreign text.

objects of their investigations. The materials published do not as yet allow us to draw conclusions concerning the absolute and relative participation of nitrogen-containing products in the formation of various groups of soil humus materials, since in most cases only qualitative and visual-semiquantitative analysis of the amino acid composition of the soils and humus materials has been performed, and since only a few works have contained quantitative determinations of the amino acid contents (in the soil samples of [7, 9, 11]; in the humic acids of various types of soils in [2, 15]; in plasma proteins and the products of the vital activity of fungi in [7, 19]). A comparison of this material from the literature is rather difficult, due to the differing conditions of chromatography used. No estimates of the quantitative contents of amino acids in other groups of soil humus, including fulvic acids, have been published.

The qualitative similarity of the amino acid composition of humus materials of different origins allowed the authors of [14] to conclude similarity of the processes of formation of various groups of humus materials, but the study of the role of amino acids in the formation of the peripheral chains of humic and fulvic acids, as well as the nitrogen-containing components of the aromatic ring, requires a detailed comparative characterization of the amino acid spectrum of all groups of soil humus and of the amino acid composition of the organic remains which arrive in the soil. /109

The present work presents the results of a quantitative analysis of the amino acid composition of the hydrolyzable (peripheral) portions of two important groups of soil humus -- the humic and fulvic acids. The preparates of humus materials were separated from samples taken in horizon A of soddy-podzolic, chernozem and sierozem soils, both virgin and cultivated. A detailed description and qualitative composition of the humus contained in the samples studied are presented in [20].

The preparates of humus materials were extracted by 0.1 n NaOH from preliminarily decalcified soil samples. After the humic acids were precipitated, the acid solution was used to separate the fulvic acids over activated carbon using the method of Forsyth [21]; the preparates of humic and fulvic acids were then subjected to dialysis. The ash content of the humic acids produced was found to be 0.5-6%; that of the fulvic acids was 6-26%. The higher ash content of the fulvic acids resulted from the presence of sodium; consequently, they actually consisted of a mixture of sodium fulvates and fulvic acids [22]. The differing contents of sodium in the fulvic acids can apparently be explained by the fact that their dialysis was performed from solutions of varying degrees of dilution. All calculations of the quantitative contents of amino acids and nitrogen performed in this work were converted to refer to the ash-free charge.

The preparates of humic and fulvic acids were hydrolyzed with 6 n HCl using a method described in a work by Kononova and Aleksandrova [14], with subsequent chromatographic determination of the amino acids. For better detection of the amino acids, a volume of hydrolysate containing no less than 70-80  $\gamma$  of amine nitrogen was applied to the chromatogram.

In this work, we used one-way ascending chromatography with n-butanol -- acetic acid -- water (4:1:5) as the system of solvents. The paper used was Leningradskaya No. 2 produced by the Volodarskiy factory. The clearest separation of the amino acids is produced by quadruple development of the chromatograms. The system of solvents which we used, however, does not allow good separation of certain amino acids; therefore, the following groups had to be determined together: aspartic acid + serine + glycine, then: glutaminic acid + threonine, valine + methionine. Development of the chromatograms was performed using a solution containing 400 mg ninhydrin + 95 ml acetone + 4 ml water + 1 ml glacial acetic acid, since this solution provided the optimal conditions for yield of the colored product [23]. The coloring is most completely developed in a dark room at room temperature over a period of 12-18 hours. Identification of the amino acids after development was performed by comparison of the amino acid spectrum of the sample being studied with a standard on the same chromatographic sheet.

Subsequently, a spot diagram was used for identification with strict observation of the same conditions of chromatography, providing for consistency in the sequence of amino acids. Before photometry, the colored products were extracted with absolute methanol containing 0.2 ml of a saturated solution of  $\text{CuSO}_4$  per 500 ml in order to increase the stability of the coloration. Test tubes containing the eluates were stored overnight in a dark room, then the intensity of the coloration was measured using a type FEK-52 photoelectric colorimeter with a number 4 light filter, except for proline, for which the number 2 filter was used.

At the same time, graduated curves were constructed for three groups of amino acids [23] and individually for proline. The experiments were repeated three to four times for each sample. The divergence between parallel determinations averaged  $\pm 5$  rel.% and did not exceed 8%. The yield of the standards averaged 95%. The quantity of nitrogen calculated according to the amino acid composition found averaged 92% for the humic acids and about 70% for the fulvic acids of the amine nitrogen determined independently by the copper method [24]. /112

The results of the quantitative determination (Tables 1 and 2) and photographs of the chromatograms (Figures 1 and 2) show qualitatively similar amino acid spectra of all the preparates investigated.

Seventeen amino acids were found, of which the following were determined in the greatest quantity in almost all samples (in order of decreasing absolute content): aspartic acid + serine + glycine, glutaminic acid + threonine,  $\alpha$ -alanine, valine and methionine, lysine and leucine, which made up approximately 80% of the entire sum of amino acids. According to the data from the literature, the hydrolysates of sixteen amino acids are generally determined in humic material by paper chromatography (using the solvent system n-butanol -- acetic acid --  $\text{H}_2\text{O}$  = 4:1:5); the acids found in most of the literature references are those in Tables 1 and 2 except for the aminobutyric acid. The content of this acid in the humic acids is not great, and the

frequent absence of the spot corresponding to this amino acid on the chromatograms can apparently be explained by the low concentration of the hydrolysate used. Scharpenseel and Krausse [2] used column chromatography to find 24 amino acids in the hydrolysates of the humic acids of certain types of soils; in addition to those listed in Tables 1 and 2, they determined methionine sulfoxide, hydroxy proline, diaminopimelic acid, isoleucine,  $\gamma$ -aminobutyric acid, methylhistidine and ornithine. Almost all of these amino acids are rare and found only for certain samples, in small quantities, apparently insufficient to be detected by paper chromatography; as concerns the isoleucine, the system of solvents which we used would not separate it from leucine.

The results produced agree well with the data from the literature, which indicate that aspartic and glutaminic acids, glycine, valine, serine, alanine, leucine and lycine are encountered in the greatest quantities. For example, the materials produced by Schaefer and presented by Kononova [25] show that the quantity of these amino acids in the soil amounts to about 83% of the entire sum of amino acids, while the cyclical amino acids make up only about 10% of the total quantity. According to Sowden [11], the corresponding figures for soil samples which he studied are approximately 90 and 7%, while Scharpenseel and Krausse [2] present figures of about 70 and 10% for humic acids, and Nikitin [15] presents figures of 80-90% and about 10% for preperates of humic acids from peaty, podzolic, chernozem and sierozem soils. However, Nikitin presented the quantitative content of only ten amino acids. An approximately similar amino acid composition was characteristic for the hydrolysate of the protein *Azotobacter*, in which the nitrogen of the amino acids listed above approaches 90% of the total nitrogen of all amino acids, while the content of nitrogen in cyclical amino acids amounts to only about 10% [7].

We know that such amino acids as aspartic and glutaminic acids, glycine, valine, alanine, leucine, serine and lycine are easily mineralized in the free state [26]. It would seem that selective enrichment of the humus with the more stable aromatic amino acids (phenylalanine, tyrosine, etc.) should occur; however, the composition of the amino acid fraction of the humus, as well as the composition of the microbe protein, contains predominately amino acids easily ammonified in the free state, which make up the most immediate source of nitrogen for plants [7]. /115

It might be assumed that the reason for the similarity of the amino acid composition of the hydrolyzable fraction of the humus material and the composition of the plasma proteins is the high mobility and dynamism of the peripheral portion of the humus compounds, which participates in constant exchange with the components of the medium and is subjected to intensive action by micro-organisms. At the same time, the specifics of humus formation leave a definite impression on the quantitative distribution of the amino acids, which is particularly evident in the rather clear difference between humic acids and fulvic acids, both in their total content of amino acids, and in their amino acid spectra.

TABLE 1. AMINO ACID COMPOSITION OF HUMIC ACIDS (in  $\mu$  mol per 1 g absolutely dry ash-free material).

| Amino Acid                     | Soddy-podzolic soil |        |               |       |            |       | Heavy Chernozem |          |        |        | Typical Stierozem |               |       |            |        |       |
|--------------------------------|---------------------|--------|---------------|-------|------------|-------|-----------------|----------|--------|--------|-------------------|---------------|-------|------------|--------|-------|
| Aspartic +<br>serine + glycine | Forest              | Fallow | Grass Mixture | Beans | Winter Rye | Corn  | Steppe          | Sainfoin | Fallow | Barley | Steppe            | Grass Mixture | Peas  | Winter Rye | Cotton | Corn  |
|                                | 238,7               | 286,8  | 288,4         | 289,4 | 204,0      | 230,0 | 182,6           | 194,3    | 186,0  | 184,0  | 260,1             | 318,0         | 326,6 | 316,5      | 308,5  | 287,8 |
| Glutaminic +<br>Threonine      | 104,5               | 112,5  | 112,5         | 109,0 | 98,2       | 102,0 | 68,9            | 71,2     | 75,1   | 66,6   | 98,5              | 109,5         | 111,9 | 98,0       | 108,9  | 108,1 |
| $\alpha$ -alanine              | 53,0                | 103,6  | 111,4         | 106,0 | 78,4       | 65,6  | 53,8            | 59,2     | 53,4   | 55,0   | 412,2             | 416,5         | 414,8 | 414,8      | 87,6   | 86,8  |
| Valine +<br>methionine         | 52,2                | 59,6   | 54,3          | 52,6  | 51,0       | 52,4  | 30,4            | 31,2     | 32,2   | 30,1   | 47,2              | 51,8          | 50,8  | 51,7       | 38,0   | 38,0  |
| Leucine                        | 44,5                | 40,8   | 57,6          | 52,3  | 48,5       | 45,7  | 25,0            | 22,9     | 29,2   | 24,1   | 48,2              | 53,5          | 51,9  | 51,9       | 53,5   | 52,2  |
| Histidine                      | 54,4                | 57,6   | 51,3          | 48,2  | 23,1       | 32,5  | 27,6            | 31,0     | 27,0   | 26,6   | 67,0              | 63,1          | 67,5  | 67,0       | 33,4   | 39,2  |
| Arginine                       | 32,1                | 32,3   | 34,4          | 34,8  | 25,4       | 27,4  | 20,8            | 20,3     | 18,7   | 19,2   | 37,1              | 34,0          | 34,2  | 38,9       | 33,6   | 29,0  |
| Aminobutyric                   | 29,4                | 28,2   | 30,4          | 30,4  | 24,0       | 26,6  | 19,3            | 19,0     | 15,7   | 15,4   | 38,0              | 34,4          | 35,3  | 35,6       | 28,3   | 28,0  |
| Proline                        | 22,0                | 16,8   | 18,4          | 19,2  | 20,6       | 20,6  | 11,9            | 13,7     | 11,9   | 13,2   | 26,8              | 24,9          | 25,4  | 26,6       | 24,5   | 23,6  |
| Tyrosine                       | 9,6                 | 12,0   | 15,0          | 20,2  | 15,6       | 10,6  | 11,5            | 13,7     | 9,6    | 11,8   | 43,7              | 42,0          | 19,8  | 18,3       | 18,6   | 18,3  |
| Phenylalanine                  | 10,9                | 7,0    | 11,4          | 16,5  | 10,7       | 7,0   | 6,1             | 6,2      | 5,4    | 5,6    | 12,0              | 12,0          | 11,1  | 7,5        | 7,1    | 7,5   |
| Cystine                        | 10,5                | 8,7    | 12,6          | 14,4  | 10,0       | 7,7   | 10,9            | 11,9     | 10,4   | 6,8    | 9,1               | 11,3          | 8,2   | 11,2       | 8,2    | 7,9   |
| Total                          | 5,8                 | 11,7   | 12,2          | 9,0   | 8,3        | 4,3   | 4,3             | 7,1      | 5,5    | 3,2    | 14,9              | 15,0          | 14,0  | 13,5       | 8,3    | 9,7   |
|                                | 667,6               | 776,6  | 809,9         | 802,0 | 627,8      | 632,4 | 470,1           | 501,7    | 480,1  | 461,6  | 784,8             | 873,8         | 871,5 | 851,5      | 760,5  | 636,1 |
| % of Primary<br>Total type     | 17,3                | 15,2   | 14,4          | 14,1  | 13,0       | 13,6  | 13,6            | 14,0     | 12,7   | 13,2   | 18,2              | 15,6          | 15,8  | 16,6       | 13,0   | 15,1  |
| amino<br>acids.                | 9,4                 | 7,7    | 9,0           | 10,7  | 9,8        | 8,2   | 10,1            | 10,4     | 9,2    | 9,6    | 9,2               | 8,8           | 8,5   | 8,9        | 8,5    | 9,8   |
| Cyclical<br>amino<br>acids.    |                     |        |               |       |            |       |                 |          |        |        |                   |               |       |            |        |       |

\* Commas indicate decimal points. -- Tr.

TABLE 2. AMINO ACID COMPOSITION OF FULVIC ACIDS (in  $\mu$  mol per 1 g absolutely dry ash-free material).

| Amino Acid                                                                                                                                                                                                                 | Soddy-podzolic soil |        |               |       |            |       | Heavy Chernozem |          |        |        | Typical Sierozem |               |       |            |        |       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|--------|---------------|-------|------------|-------|-----------------|----------|--------|--------|------------------|---------------|-------|------------|--------|-------|
| Aspartic +<br>serine + glycine<br>Glutaminic +<br>Threonine<br>$\alpha$ -alanine<br>Valine +<br>methionine<br>Leucine<br>Histidine<br>Arginine<br>Aminobutyric<br>Proline<br>Tyrosine<br>Phenylalanine<br>Cystine<br>Total | Forest              | Fallow | Grass Mixture | Beans | Winter Rye | Corn  | Steppe          | Sainfoin | Fallow | Barley | Steppe           | Grass Mixture | Peas  | Winter Rye | Cotton | Corn  |
|                                                                                                                                                                                                                            | 254,0               | 248,5  | 270,0         | 280,5 | 260,0      | 260,1 | 192,1           | 189,0    | 186,8  | 176,0  | 192,0            | 214,8         | 212,0 | 210,8      | 191,5  | 182,6 |
|                                                                                                                                                                                                                            | 105,2               | 124,8  | 128,5         | 120,0 | 124,8      | 126,2 | 112,5           | 112,7    | 105,5  | 98,8   | 107,5            | 109,5         | 113,0 | 104,2      | 105,2  | 106,1 |
|                                                                                                                                                                                                                            | 52,8                | 47,2   | 53,5          | 52,6  | 48,5       | 44,7  | 42,1            | 42,5     | 39,4   | 37,6   | 49,3             | 49,8          | 51,5  | 46,5       | 44,7   | 45,0  |
|                                                                                                                                                                                                                            | 42,3                | 44,2   | 47,0          | 45,0  | 43,5       | 46,0  | 36,5            | 36,0     | 31,2   | 35,4   | 37,5             | 37,6          | 39,2  | 39,8       | 38,0   | 36,7  |
|                                                                                                                                                                                                                            | 22,7                | 18,1   | 40,7          | 18,7  | 20,0       | 21,2  | 21,6            | 22,9     | 21,3   | 20,4   | 23,3             | 23,8          | 23,3  | 24,0       | 22,8   | 20,2  |
|                                                                                                                                                                                                                            | 10,1                | 10,5   | 10,4          | 10,2  | 9,7        | 9,8   | 8,8             | 8,5      | 8,5    | 8,2    | 11,5             | 12,0          | 11,2  | 10,8       | 10,0   | 10,1  |
|                                                                                                                                                                                                                            | 6,6                 | 7,1    | 7,6           | 7,7   | 7,7        | 7,4   | 7,0             | 6,6      | 6,8    | 7,4    | 8,5              | 8,9           | 8,4   | 8,3        | 8,2    | 8,3   |
|                                                                                                                                                                                                                            | 4,7                 | 5,1    | 4,5           | 3,2   | 3,9        | 4,3   | 3,1             | 2,8      | 2,2    | 2,6    | 4,5              | 4,8           | 5,5   | 4,9        | 5,1    | 7,1   |
|                                                                                                                                                                                                                            | 23,9                | 26,2   | 28,9          | 25,9  | 25,0       | 29,0  | 16,5            | 18,0     | 19,2   | 17,9   | 30,0             | 27,7          | 29,1  | 27,5       | 30,2   | 34,2  |
| 6,4                                                                                                                                                                                                                        | 9,1                 | 9,1    | 8,1           | 6,8   | 5,6        | 10,5  | 7,8             | 7,8      | 9,1    | 9,1    | 9,9              | 10,4          | 8,5   | 9,1        | 8,3    |       |
| 4,0                                                                                                                                                                                                                        | 8,4                 | 8,2    | 8,2           | 7,3   | 5,4        | 6,3   | 5,4             | 4,4      | 4,7    | 4,1    | 7,3              | 5,4           | 5,4   | 4,5        | 4,1    |       |
| 3,7                                                                                                                                                                                                                        | 4,7                 | 4,9    | 4,7           | 3,8   | 4,1        | 8,2   | 8,2             | 5,9      | 4,3    | 4,3    | 4,8              | 4,9           | 4,8   | 3,9        | 3,8    |       |
| 6,1                                                                                                                                                                                                                        | 9,4                 | 11,1   | 12,5          | 9,5   | 8,3        | 9,6   | 8,5             | 9,9      | 7,7    | 8,8    | 13,5             | 12,0          | 10,1  | 8,8        | 8,3    |       |
| 545,5                                                                                                                                                                                                                      | 563,3               | 603,4  | 597,3         | 570,5 | 571,9      | 472,1 | 468,9           | 450,2    | 430,1  | 493,2  | 524,9            | 524,0         | 505,3 | 480,8      | 473,0  |       |
| % of<br>Total<br>Primary<br>type<br>amino<br>acids.<br>Cyclical<br>amino                                                                                                                                                   | 3,9                 | 4,0    | 3,7           | 3,5   | 3,7        | 3,8   | 4,0             | 3,8      | 3,9    | 4,2    | 5,0              | 4,3           | 4,8   | 4,8        | 4,8    | 5,4   |
|                                                                                                                                                                                                                            | 2,6                 | 4,0    | 3,7           | 3,5   | 3,1        | 2,6   | 4,8             | 4,6      | 4,3    | 4,2    | 3,7              | 4,3           | 3,6   | 3,6        | 3,4    | 3,0   |

\* Commas indicate decimal points.

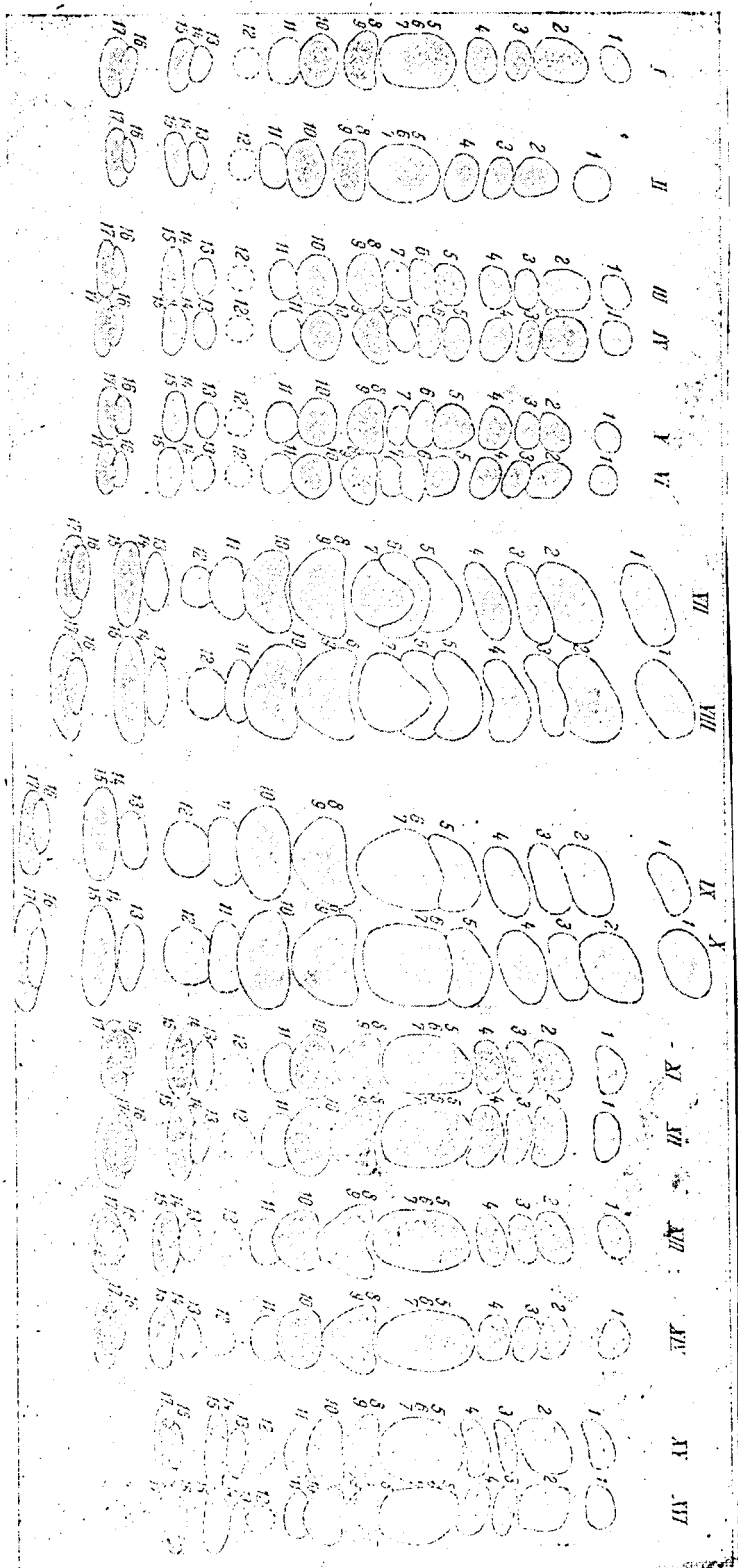


Figure 1. Chromatogram of Humic Acid Hydrolysates: Key: a, Soddy podzolic soil (I-IV): I, Beneath forest; II, In fallow; III, Beneath grass mixture; IV, Beneath peas; V, Beneath winter rye; VI, Beneath corn; b, Heavy black earth (VII-X): VII, Beneath steppe; VIII, Beneath sainfoin; IX, In fallow; X, Beneath barley; c, Typical sierozem (XI-XVI): XI, Beneath steppe; XII, Beneath grass mixture; XIII, Beneath peas; XIV, Beneath winter rye; XV, Beneath cotton; XVI, Beneath corn. 1, Cystine; 2, Lysine; 3, Histidine; 4, Arginine; 5, 6, 7, Aspartic, serine, glycine; 8, 9, Glutamic, threonine; 10,  $\alpha$ -alanine; 11, Proline; 12, Aminobutyric; 13, Tyrosine; 14, 15, Valine methionine; 16, Phenylalanine; 17, Leucine

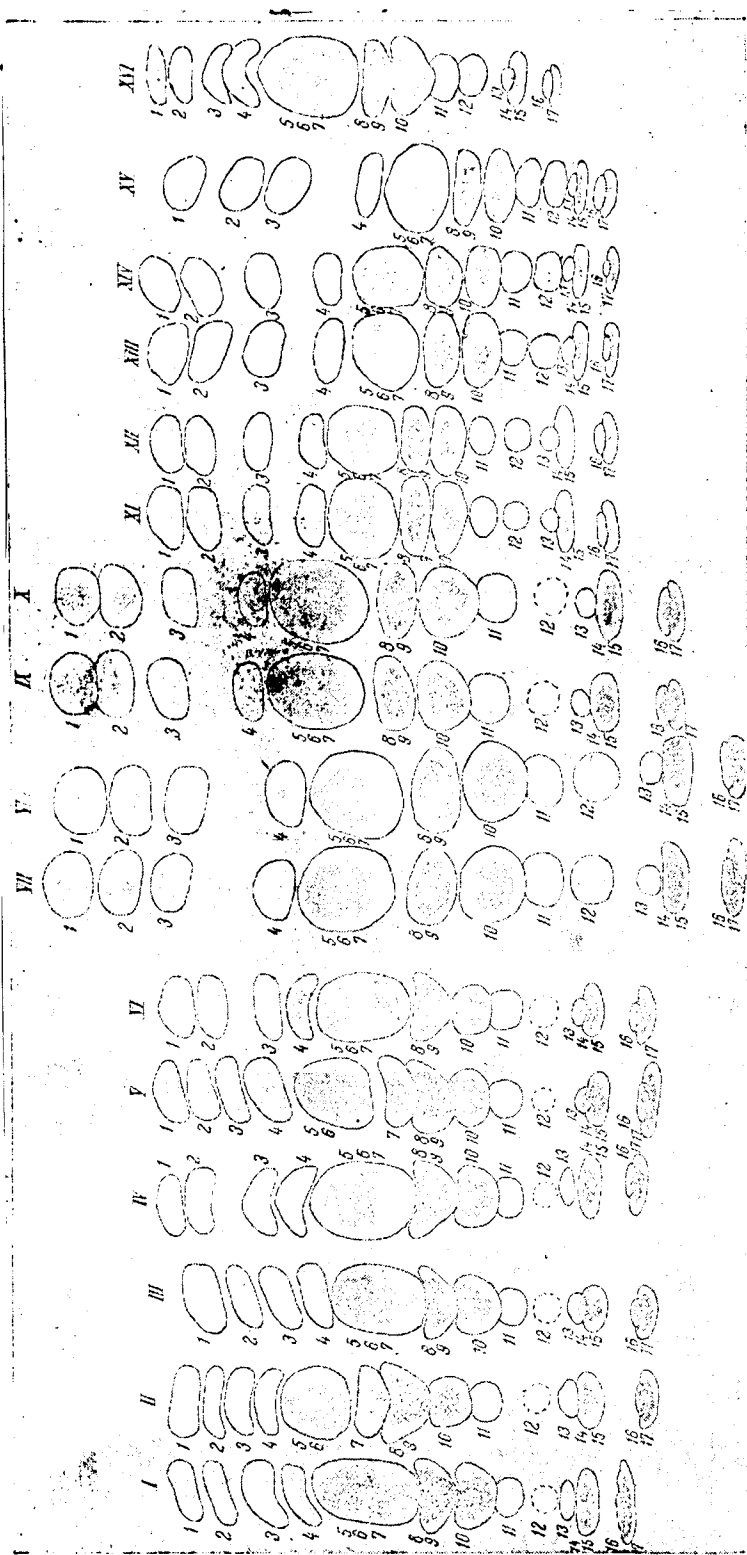


Figure 2. Chromatogram of Hydrolysates of Fulvic Acids: a, Soddy podzolic soil (I-VI): 1, Beneath forest; II, In fallow; III, Beneath grass mixture; IV, Beneath beans; V, Beneath winter rye; VI, Beneath corn; b, Heavy black earth (VII-X): VII, In steppe; VIII, Beneath sainfoin; IX, In fallow; X, Beneath barley; c, Typical sierozem (XI-XVI): XI, In steppe; XII, Beneath grass mixture; XIII, Beneath peas; XIV, Beneath winter rye; XV, Beneath cotton; XVI, Beneath corn. 1, Cystine; 2, Lysine; 3, Histidine; 4, Arginine; 5, 6, 7, Aspartic, serine, glycine; 8, 9, Glutamic, threonine; 10,  $\alpha$ -alanine; 11, Proline; 12, Aminobutyric; 13, Tyrosine; 14, 15, Valine, methionine; 16, Phenylalanine; 17, Leucine

TABLE 3. SUMMARY CONTENT OF AMINO ACIDS AND NITROGEN OF AMINO ACIDS  
IN HUMIC ACIDS AND FULVIC ACIDS

| Soil<br>Area<br>and<br>Plant<br>Type | Humic Acids                     |                                |                                       |                                 |                                 | Fulvic Acids                             |                                |                                       |                                            |                        |
|--------------------------------------|---------------------------------|--------------------------------|---------------------------------------|---------------------------------|---------------------------------|------------------------------------------|--------------------------------|---------------------------------------|--------------------------------------------|------------------------|
|                                      | Contents of amino<br>acids in % | Content of amino<br>acids in % | Content of nitrogen<br>acids in %     | Contents of amino<br>Acids in % | Content of amino<br>acids in %  | Contents of amino<br>Acids in %          | Content of amino<br>acids in % | Content of Nitrogen<br>in Fulvic Acid | Of Nitrogen in Hydro-<br>lyzable Compounds |                        |
| Soddy-<br>podzolic                   | Of Soil                         | Of Humic Acid                  | Of Hydrolyzable Part<br>of Humic Acid | Of Total Soil Nitrogen          | Of Total Humic Acid<br>Nitrogen | Of Nitrogen in<br>Hydrolyzable Compounds | Of Soil                        | Of Fulvic Acid                        | Of Hydrolyzable<br>Portion of Fulvic Acid  | Of Total Soil Nitrogen |
|                                      | Forest<br>Fallow                | 8,29                           | 18,6                                  | 2,97                            | 25,6                            | 38,0                                     | 0,060                          | 6,26                                  | 8,15                                       | 4,08                   |
|                                      | Grass Mix.                      | 9,45                           | 20,6                                  | 2,97                            | 33,2                            | 48,8                                     | 0,057                          | 6,67                                  | 8,89                                       | 4,70                   |
|                                      | Beans                           | 9,93                           | 24,4                                  | 3,71                            | 30,5                            | 50,7                                     | 0,043                          | 7,11                                  | 9,46                                       | 3,84                   |
|                                      | Winter rye                      | 9,83                           | 23,9                                  | 2,63                            | 28,6                            | 46,2                                     | 0,039                          | 7,04                                  | 9,35                                       | 2,97                   |
| Heavy<br>Black<br>Earth              | Corn                            | 7,69                           | 17,5                                  | 3,34                            | 33,0                            | 50,6                                     | 0,040                          | 6,72                                  | 8,47                                       | 5,92                   |
|                                      |                                 | 0,020                          | 17,2                                  | 1,78                            | 27,0                            | 40,0                                     | 0,039                          | 6,73                                  | 8,42                                       | 4,67                   |
|                                      | Steppe<br>Sainfoin              | 0,127                          | 20,8                                  | 4,74                            | 22,2                            | 49,2                                     | 0,061                          | 5,66                                  | 7,81                                       | 2,02                   |
|                                      | Fallow                          | 0,141                          | 21,8                                  | 4,09                            | 21,6                            | 48,8                                     | 0,066                          | 5,60                                  | 7,73                                       | 1,64                   |
|                                      | Barley                          | 0,130                          | 18,2                                  | 3,96                            | 21,7                            | 42,2                                     | 0,060                          | 5,47                                  | 7,46                                       | 1,47                   |
| Typical<br>Sierozem                  | Steppe<br>Grass Mix.            | 0,096                          | 19,0                                  | 3,16                            | 22,1                            | 45,0                                     | 0,077                          | 5,12                                  | 7,03                                       | 2,09                   |
|                                      | Peas                            | 9,63                           | 25,3                                  | 3,20                            | 29,6                            | 50,0                                     | 0,017                          | 5,83                                  | 8,15                                       | 2,01                   |
|                                      | Cotton                          | 10,46                          | 26,2                                  | 3,62                            | 32,1                            | 56,0                                     | 0,019                          | 6,28                                  | 8,27                                       | 1,32                   |
|                                      |                                 | 10,57                          | 23,8                                  | 3,21                            | 34,4                            | 56,5                                     | 0,024                          | 6,24                                  | 8,66                                       | 2,19                   |
|                                      | Corn                            | 10,32                          | 25,2                                  | 3,07                            | 30,7                            | 48,6                                     | 0,025                          | 6,00                                  | 8,35                                       | 3,10                   |
| Typical<br>Sierozem                  | Steppe<br>Grass Mix.            | 0,027                          | 24,6                                  | 3,21                            | 31,4                            | 48,7                                     | 0,021                          | 5,71                                  | 7,93                                       | 2,35                   |
|                                      | Peas                            | 8,86                           | 21,1                                  | 2,42                            | 30,0                            | 46,7                                     | 0,014                          | 5,62                                  | 7,22                                       | 2,55                   |
|                                      | Cotton                          | 9,63                           | 25,3                                  | 3,20                            | 29,6                            | 50,0                                     | 0,017                          | 5,83                                  | 8,15                                       | 2,01                   |
|                                      |                                 | 10,46                          | 26,2                                  | 3,62                            | 32,1                            | 56,0                                     | 0,019                          | 6,28                                  | 8,27                                       | 1,32                   |
|                                      | Corn                            | 10,57                          | 23,8                                  | 3,21                            | 34,4                            | 56,5                                     | 0,024                          | 6,24                                  | 8,66                                       | 2,19                   |

The amino acids bonded in the humus material take on a certain stability, apparently making possible the "selection" of certain amino acids and their subsequent condensation in the ring. These bonded amino acids are contained in the hydrolyzable portion of the humus material in very small quantities, or are not detectable at all (for example tryptophan). Their participation in the formation of the ring is revealed, for example, by the data of Flaig [27], who discovered humic acid with an alkaline compound of the indol carbonic acid type in the products of melting of the nonhydrolyzable portion of the remains which, in his opinion, could have been formed under these conditions only from tryptophan. When he melted the nonhydrolyzable soil residue with KOH, Shmuk [28] detected an odor of skatole which, as we know, is also a product of the decomposition of tryptophan.

It is also interesting to note the correlation which is observed according to our data between the content of cyclic amino acids in the hydrolysates of humic and fulvic acids and the degree of condensation of these groups of humus materials.

The humic acids from various types of soils differ notably in their absolute contents of amino acids (Tables 1, 3). The richest in humic acids are the sierozems, where the total humic acid content amounts to 636-874  $\mu\text{mol}$  per 1 g of material, or 8.8-10.5% of the weight of the humic acid; the least content of amino acids in the humic acids is that of the chernozems: 460-500  $\mu\text{mol}$  (5.5-6.1%); the humic acids of soddy-podzolic soils contain 628-809  $\mu\text{mol}$  amino acids per 1 g material (7.6-9.9%). The content of amino acids, like the content of the hydrolyzable and nonhydrolyzable portions in the molecules of the humic acids, is determined primarily by the nature of the humification, which is specific for each type of soil; also, the literature [2] contains indications that the highest sum of amino acids is observed in soils with retarded humification, enriched in semidecomposed protein material. According to our data, the absolute content of amino acids in humus materials results not only from the conditions of the process of humification, but also the nature of the particles of these compounds themselves. Under the conditions present in the chernozem zone, where phenomena of synthesis predominate and the most highly condensed humic acids are formed, the content of side chains in the molecules and consequently the quantity of amino acids in the hydrolysate, calculated per gram of humic acid, is considerably less than the sum of amino acids in the less highly condensed humic acids of the soddy-podzolic and sierozem soils.

The fulvic acids of various types of soils differ to a lesser extent in their absolute content of amino acids (Tables 2, 3). The fulvic acids of the soddy-podzolic and sierozem soils were found to contain 473-603  $\mu\text{mol}$  amino acids per gram of preparate, while the fulvic acids of chernozems contained somewhat less, 430-473  $\mu\text{mol}$ , the fulvic acids being more homogeneous than the other soil humus bodies as concerns this characteristic as well.

The regularities which we are discussing concerning the amino acid content as a function of the type of soil relate only to groups of humic and fulvic acids, while the total sum of amino acids calculated in relation to the

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soil as a whole changes differently. the maximum reserve of amino acids in humus materials is characteristic for chernozem soils, exceeding the quantity of amino acids in soddy podzolic and chernozem soils by two to three times (Table 3).

Within each of these types of soils, the fluctuations in the total sum of amino acids in the humus material fraction occur due to a smooth increase or decrease of the content of all amino acids detected, with slight exceptions which, however, do not exceed the limits of error of our experiments, resulting from the nature and, more importantly, the intensity of the biochemical processes occurring beneath different plant types. Some increase in the total sum of amino acids in humic acids taken from soils in which bean crops are grown has been noted; here, doubtless, there is more protein material and the microbiological activity is more intensive and varied.

Our attention is drawn by the very close relative contents of amino acids within the hydrolyzable compounds of humic and fulvic acids from various types of soils (Table 3) amounting in the hydrolysate of humic acid to 20-25% of all of the compounds which go over into solution. The role of amino acids in the nitrogen balance of the humus is even more significant. The calculations presented in this same table show that the amino acids take active part in the formation of humus material, being one of the primary sources of nitrogen resulting from humification: the nitrogen of amino acids makes up half or more of all the nitrogen in the hydrolyzable humic acid compounds and 26-38% of the hydrolyzable compounds of nitrogen in fulvic acids. The remaining portion of the nitrogen in the hydrolyzable compounds is primarily present as amides and amino sugars.

A comparison of the two groups of humus materials indicates the great degree of participation of amino acids and the construction of the hydrolyzable portion of humic acids, in comparison with fulvic acids.

The differences between the humic and fulvic acids are observed not only in their absolute quantity but in the relative content of the individual amino acids or groups of acids. In humic acids, the primary type amino acid fraction amounts to 13-18% of the total sum, the cyclical amino acids amount to 8-11%; in fulvic acids, the figures are 3.5-5 and 3-5% respectively. An analogous conclusion concerning the relationships of these two groups can be drawn from the table showing a visual estimate of the amino acid composition of humus material presented by Kononova and Aleksandrova [14].

The share of such groups of amino acids as aspartic acid + serine + glycine and glutaminic acid + threonine, i.e. the groups containing the acid amino acids, amounts to 60-70% of the entire sum for fulvic acids, but only half for humic acids. The relationships of the sum of threonine and glutaminic acid and  $\alpha$ -alanine change rather regularly. With a relatively high content of the group consisting of glutaminic acid + threonine in the fulvic acids, the quantity of  $\alpha$ -alanine is generally low. In humic acids, the relative contents of these amino acids are approximately identical, although if a reduction in the share of glutaminic acid and threonine occurs, the content

of  $\alpha$ -alanine may increase, so that the sum of these amino acids remains almost unchanged. We know that  $\alpha$ -alanine can be formed by transamination from glutaminic acid [29]; the possibility cannot be excluded that this process occurs during the formation of humic acids, particularly in sierozem soils beneath beans and grain cultures, which show an increased content of  $\alpha$ -alanine with a slightly reduced content of glutaminic acid. In fulvic acids, the content of aminobutyric acid is slightly increased; according to Scharpenseel and Krausse [2], this content depends strongly on the microbiological activity. /118

As concerns the relative contents of the remaining amino acids: cystine, valine + methionine, leucine -- the differences between the humic and fulvic acids are quite insignificant, irregular, and generally within the limits of error of the determinations.

The differences detected in the contents of individual amino acids or groups of amino acids are not random; they are related to the mechanism of humus formation and explain some of the chemical properties of the humus materials. The content of cyclical amino acids and primary type amino acids in fulvic acids, which is two to three times less than their content in the humic acids, as well as the slightly greater content of acid amino acids and aminobutyric acid in the fulvic acids agree with the more acid nature of these compounds, their lower degree of condensation, their greater migration capacity and greater vulnerability to the action of micro-organisms. Keeping in mind the indications of the literature [25] concerning the genetic relationship between humic acids and fulvic acids, we might assume that one of the most important conditions of humification and the formation of humic acids is the relative accumulation of amino acids, especially cyclical type amino acids, which participate actively in condensation reactions. /119

The amino acid spectra of humic acids in various types of soils differ quite insignificantly, the differences found beneath identical crops in different types of soils being less than the differences found in the same types of soils but beneath different crops. The differences in this case as well are observed primarily for the group consisting of glutaminic acid + threonine, as well as for  $\alpha$ -alanine, i.e. the same regularity is repeated which was noted in the comparison of humic acids and fulvic acids. In humic acids contained in soddy-podzolic soils and sierozem beneath intertilled crops in some cases the content of  $\alpha$ -alanine is decreased and the quantity of glutaminic acid increased.

As a whole, the amino acid spectra of humic acids from different soils are similar and depend comparatively little on the nature of the vegetation growing in the soil, which can be seen quite clearly for the chernozem soils (Figure 3). The amino acid spectra of all of the fulvic acids studied are even more similar.

The identity of the amino acid spectra of the humus materials from various types of soils allows us to conclude that the principle and nature of the combination of amino acids in the hydrolyzable portion of each of these groups of humus materials are basically identical, and that their composition

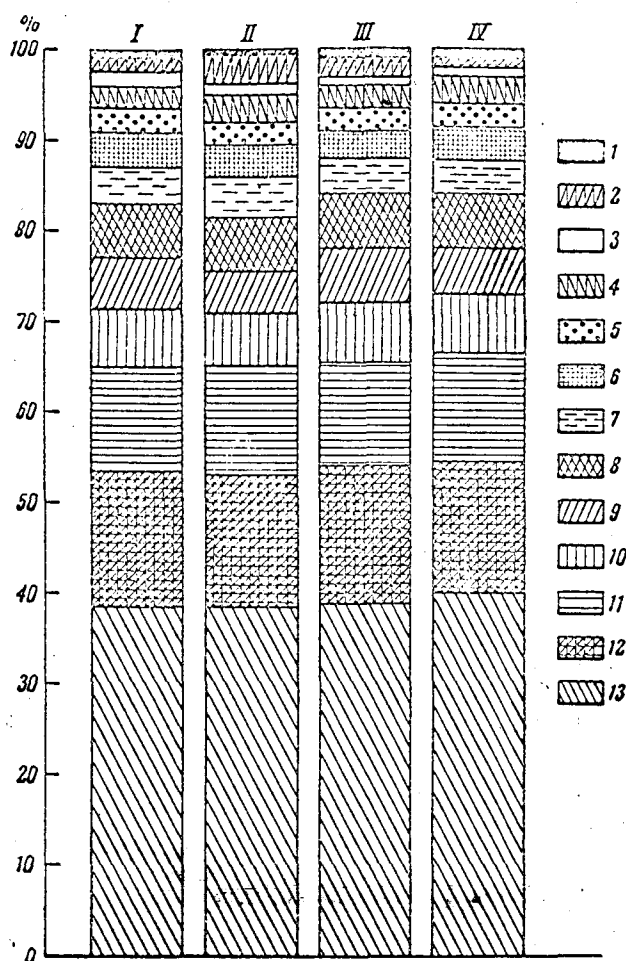


Figure 3. Relative Content of Amino Acids in Humic Acids of Chernozems (percent of total sum): I, In steppe; II, Beneath sainfoin; III, In fallow; IV, Beneath barley. 1, Cystine; 2, Phenylalanine; 3, Tyrosine; 4, Proline; 5, Aminobutyric; 6, Arginine; 7, Histidine; 8, Lycine; 9, Leucine; 10, Valine + methionine; 11,  $\alpha$ -alanine; 12, Glutaminic + threonine; 13, Aspartic + serine + glycine

3. The composition of the amino acids in the hydrolyzable portion of humus materials consists of predominantly: aspartic acid + serine + glycine, glutaminic acid + threonine,  $\alpha$ -alanine, valine + methionine, lycine, leucine making up approximately 80% of the entire sum of amino acids.

is regulated by the nature and mechanism of the reactions involved in the formation of the humus materials (humic acids or fulvic acids) depending only very slightly on the general conditions of soil formation even across a broad range of such basically different soil types as chernozem, sierozem and soddy-podzolic soil.

### Conclusions

1. The relative contents of groups of amino acids and their individual representatives in the composition of humic materials of soddy-podzolic, chernozem and sierozem soils are quite similar, indicating identity in the structure of the hydrolyzable portions of the humic acids and fulvic acids, regardless of the type of soil.

2. The hydrolysates of humic and fulvic acids contained seventeen amino acids; the humic acids contain 460-870  $\mu\text{mol}$  amino acids per gram (5.5-10.5%), while the fulvic acids contain 430-600  $\mu\text{mol}$  per gram, or 5.1-7.1%. On the average, 22% of the hydrolyzable compounds of the humic acids and 8% of the hydrolyzable compounds of the fulvic acids are amino acids.

4. The total quantity of amino acids and the relative content of the cyclical forms correlate well with the degree of condensation of the humus materials. The amino acids are more significant in the construction of the humic acids than in the construction of the fulvic acids. The fulvic acids differ from the humic acids in that their content of cyclic amino acids and primary type amino acids is considerably lower.

5. The nitrogen of amino acids, calculated according to the content of individual compounds, amounts to 40-50% of all the hydrolyzable forms of nitrogen compounds in the humic acids and 30-40% in the fulvic acids, which indicates the importance of the role of amino acids in the formation of humus materials. /120

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